

It is not believed that extensions of time or fees for net addition of claims are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 19-0036.

Amendments

In the Claims:

Please cancel claim 79 without prejudice or disclaimer.

Please substitute the following claim 75 for the pending claim 75:

75. (Twice amended) A DNA molecule which encodes an RNA molecule comprising:

- (a) at least one *cis*-acting sequence element,
- (b) a first open reading frame which encodes a non-cytopathic temperature-sensitive alphaviral replicase, wherein non-cytopathicity and temperature sensitivity are conferred by one or more mutations in the genes encoding the nonstructural proteins of said replicase, and
- (c) at least one second nucleotide sequence selected from the group consisting of:
 - (i) a second open reading frame encoding a protein, or portion thereof, wherein said second open reading frame is in a

translatable format after one or more RNA-dependent RNA replication events;

- (ii) a sequence complementary to all or part of the second open reading frame of (i); and
- (iii) a sequence encoding an untranslated RNA molecule, or complement thereof;

wherein said second nucleotide sequence is operably linked to a promoter which is recognized by said non-cytopathic, temperature-sensitive alphaviral replicase.

Please substitute the following claim 81 for the pending claim 81:

81. (Twice amended) The DNA molecule of claim 75, wherein said alphaviral replicase is derived from a Sindbis virus.

Please substitute the following claim 82 for the pending claim 82:

82. (Once amended) The DNA molecule of claim 75 which encodes an alphaviral replicase having replicase activity at 34°C which is at least five fold lower than the replicase activity exhibited at 29°C.

Please substitute the following claim 86 for the pending claim 86:

86. (Once amended) A method of making a recombinant host cell comprising introducing the DNA molecule of claim 75 into a host cell *in vitro*.

Please substitute the following claim 93 for the pending claim 93:

93. (Once amended) A method for producing a protein or an untranslated RNA molecule in a recombinant host cell comprising:

- (a) introducing at least one DNA molecule of claim 75 into said host cells *in vitro*;
- (b) culturing said host cells under conditions suitable for expression of said protein or untranslated RNA molecule; and
- (c) recovering said protein or untranslated RNA molecule;

wherein said protein or untranslated RNA molecule is encoded by said DNA molecule.

Please substitute the following claim 94 for the pending claim 94:

94. (Once amended) A method for producing a protein or an untranslated RNA molecule in a recombinant host cell comprising:

- (a) introducing at least one RNA molecule of claim 90 into said host cells *in vitro*;
- (b) culturing said host cells under conditions suitable for expression of said protein or untranslated RNA molecule; and
- (c) recovering said protein or untranslated RNA molecule;

wherein said protein or untranslated RNA molecule is encoded by said RNA molecule.

Please substitute the following claim 97 for the pending claim 97:

97. (Once amended) A method for producing alphaviral particles, said method comprising:

- (a) introducing into a host cell *in vitro* at least one DNA molecule of claim 75 having one or more open reading frames which encode alphaviral structural proteins;
- (b) growing host cells under culture conditions suitable for the production of alphaviral particles which contain an RNA transcription product of said DNA molecule; and
- (c) recovering said alphaviral particles.

Please substitute the following claim 98 for the pending claim 98:

98. (Once amended) A method for producing a protein encoded by RNA contained in an alphaviral particle produced by the method of claim 97 in a recombinant host cell comprising:

- (a) infecting a host cell *in vitro* with the alphaviral particle;

- (b) growing said host cell under culture conditions suitable for the production of said protein; and
- (c) recovering said protein.

Please substitute the following claim 100 for the pending claim 100:

100. (Once amended) A method for regulating the expression of a protein or an untranslated RNA molecule in a recombinant host cell comprising:

- (a) growing host cells under suitable culture conditions;
- (b) introducing at least one DNA molecule of claim 75 into said host cells *in vitro*; and
- (c) changing the temperature of the host cell culture from:
 - (i) a permissive temperature to a restrictive temperature, or
 - (ii) a restrictive temperature to a permissive temperature;

wherein said protein or untranslated RNA molecule is encoded by said DNA molecule.

Please substitute the following claim 101 for the pending claim 101:

101. (Once amended) A method for regulating the expression of a protein or an untranslated RNA molecule in a recombinant host cell comprising:

- (a) growing host cells under suitable culture conditions;
- (b) introducing at least one RNA molecule of claim 90 into said host cells *in vitro*; and
- (c) changing the temperature of the host cell culture from:
 - (i) a permissive temperature to a restrictive temperature, or
 - (ii) a restrictive temperature to a permissive temperature;

wherein said protein or untranslated RNA molecule is encoded by said RNA molecule.

Please substitute the following claim 103 for the pending claim 103:

103. (Twice amended) A DNA vector system comprising one or more

polynucleotides which encode RNA molecules, said RNA molecules comprising:

- (a) at least one *cis*-acting sequence element,
- (b) a first open reading frame having a nucleotide sequence encoding a non-cytopathic, temperature-sensitive alphaviral replicase, wherein non-cytopathicity and temperature sensitivity are conferred by one or more mutations in the genes encoding the nonstructural proteins of said replicase, and
- (c) at least one second nucleotide sequence selected from the group consisting of:
 - (i) a second open reading frame encoding a protein, or portion thereof, wherein said second open reading frame is in a translatable format after one or more RNA-dependent RNA replication events;
 - (ii) a sequence complementary to all or part of the second open reading frame of (i); and
 - (iii) a sequence encoding an untranslated RNA molecule, or complement thereof;

wherein said second nucleotide sequence is operably linked to a promoter which is recognized by said non-cytopathic, temperature-sensitive alphaviral replicase.

Please substitute the following claim 105 for the pending claim 105:

105. (Once amended) The DNA vector system of claim 103 which encodes an alphaviral replicase having replicase activity at 34°C which is at least five fold lower than the replicase activity exhibited at 29°C.

Please substitute the following claim 109 for the pending claim 109:

109. (Once amended) A method of making a recombinant host cell comprising introducing at least one polynucleotide of claim 103 into a host cell *in vitro*.

Please substitute the following claim 116 for the pending claim 116:

116. (Once amended) A method for producing a protein or an untranslated RNA molecule in a recombinant host cell comprising:

- (a) growing host cells under suitable culture conditions;
- (b) introducing at least one DNA molecule of claim 103 into said host cells *in vitro*;
- (c) recovering said protein or untranslated RNA molecule;

wherein said protein or untranslated RNA molecule is encoded by said DNA molecule.

Please substitute the following claim 117 for the pending claim 117:

117. (Once amended) A method for producing a protein or an untranslated RNA molecule in a recombinant host cell comprising:

- (a) growing host cells under suitable culture conditions;
- (b) introducing at least one RNA molecule of claim 113 into said host cells *in vitro*; and
- (c) recovering said protein or untranslated RNA molecule;

wherein said protein or untranslated RNA molecule is encoded by said RNA molecule.

Please substitute the following claim 120 for the pending claim 120:

120. (Once amended) A method for producing an alphaviral particle comprising:

- (a) growing host cells under suitable culture conditions;
- (b) introducing into said host cells *in vitro* at least one DNA molecule of claim 103 having one or more open reading frames which encode alphaviral structural proteins;
- (c) producing an alphaviral particle; and
- (d) recovering said alphaviral particle.

Please substitute the following claim 121 for the pending claim 121:

121. (Once amended) A method for producing a protein in a recombinant host cell comprising:

- (a) growing host cells under suitable culture conditions;
- (b) infecting said host cells *in vitro* with an alphaviral particle produced by the method of claim 120; and
- (c) recovering said protein;

wherein said protein is encoded by nucleic acid contained in said alphaviral particle.

Please substitute the following claim 123 for the pending claim 123:

123. (Once amended) A method for regulating the expression of a protein or an untranslated RNA molecule in a recombinant host cell comprising:

- (a) growing host cells under suitable culture conditions;
- (b) introducing at least one DNA molecule of claim 103 into said host cells *in vitro*; and
- (c) changing the temperature of the host cell culture from:
 - (i) a permissive temperature to a restrictive temperature, or
 - (ii) a restrictive temperature to a permissive temperature;

wherein said protein or untranslated RNA molecule is encoded by said DNA molecule.

Please substitute the following claim 124 for the pending claim 124:

124. (Once amended) A method for regulating the expression of a protein or an untranslated RNA molecule in a recombinant host cell comprising:

- (a) growing host cells under suitable culture conditions;
- (b) introducing at least one RNA molecule of claim 111 into said host cells *in vitro*; and
- (c) changing the temperature of the host cell culture from:
 - (i) a permissive temperature to a restrictive temperature, or
 - (ii) a restrictive temperature to a permissive temperature;

wherein said protein or untranslated RNA molecule is encoded by said RNA molecule.

Please substitute the following claim 125 for the pending claim 125:

125. (Twice amended) A composition comprising one or more RNA molecules, said RNA molecules comprising:

- (a) at least one *cis*-acting sequence element,
- (b) a first open reading frame having a nucleotide sequence encoding a non-cytopathic, temperature-sensitive alphaviral replicase, wherein non-cytopathicity and temperature sensitivity are conferred by one or more mutations in the genes encoding the nonstructural proteins of said replicase, and
- (c) at least one second nucleotide sequence selected from the group consisting of:
 - (i) a second open reading frame encoding a protein, or portion thereof, wherein said second open reading frame is in a translatable format after one or more RNA-dependent RNA replication events;
 - (ii) a sequence complementary to all or part of the second open reading frame of (i); and
 - (iii) a sequence encoding an untranslated RNA molecule, or complement thereof;

wherein said second nucleotide sequence is operably linked to a promoter which is activated by said non-cytopathic, temperature-sensitive alphaviral replicase.

Please substitute the following claim 126 for the pending claim 126:

126. (Once amended) The DNA molecule of claim 75, wherein said alphaviral replicase is derived from a Semliki Forest Virus.

Please substitute the following claim 127 for the pending claim 127:

127. (Once amended) The DNA molecule of claim 75, wherein said alphaviral replicase is derived from an Aura virus.

Please substitute the following claim 128 for the pending claim 128:

128. (Once amended) The DNA molecule of claim 75, wherein said alphaviral replicase is derived from a virus selected from the group consisting of Bebaru virus, Cabassou virus, Chikungunya virus, Eastern equine encephalomyelitis virus, Fort Morgan virus, Getah virus, Kyzylagach virus, Mayoaro virus, Middleburg virus, Mucambo virus, Ndumu virus, Pixuna virus, Tonate virus, Trinita virus, Una virus, Western equine encephalomyelitis virus, Whataroa virus, Venezuelan equine encephalomyelitis virus (VEE), and Ross River virus.

Please substitute the following claim 129 for the pending claim 129:

129. (Once amended) The DNA vector system of claim 103, wherein said alphaviral replicase is derived from a Sindbis virus.

Please substitute the following claim 130 for the pending claim 130:

130. (Once amended) The DNA vector system of claim 103, said alphaviral replicase is derived from a Semliki Forest Virus.

Please substitute the following claim 131 for the pending claim 131:

131. (Once amended) The DNA vector system of claim 103, said alphaviral replicase is derived from an Aura virus.

Please substitute the following claim 132 for the pending claim 132:

132. (Once amended) The DNA vector system of claim 103, wherein said alphaviral replicase is derived from a virus selected from the group consisting of Bebaru virus, Cabassou virus, Chikungunya virus, Eastern equine encephalomyelitis virus, Fort Morgan virus, Getah virus, Kyzylagach virus, Mayoaro virus, Middleburg virus, Mucambo

virus, Ndumu virus, Pixuna virus, Tonate virus, Trinita virus, Una virus, Western equine encephalomyelitis virus, Whataroa virus, Venezuelan equine encephalomyelitis virus (VEE), and Ross River virus.

Please substitute the following claim 133 for the pending claim 133:

133. (Once amended) The RNA molecule of claim 125, wherein said alphaviral replicase is derived from a Sindbis virus.

Please substitute the following claim 134 for the pending claim 134:

134. (Once amended) The RNA molecule of claim 125, wherein said alphaviral replicase is derived from a Semliki Forest Virus.

Please substitute the following claim 135 for the pending claim 135:

135. (Once amended) The RNA molecule of claim 125, wherein said alphaviral replicase is derived from an Aura virus.

Please substitute the following claim 136 for the pending claim 136:

136. (Once amended) The RNA molecule of claim 125, wherein said alphaviral replicase is derived from a virus selected from the group consisting of Bebaru virus, Cabassou virus, Chikungunya virus, Eastern equine encephalomyelitis virus, Fort Morgan virus, Getah virus, Kyzylagach virus, Mayoaro virus, Middleburg virus, Mucambo virus, Ndumu virus, Pixuna virus, Tonate virus, Trinita virus, Una virus, Western equine encephalomyelitis virus, Whataroa virus, Venezuelan equine encephalomyelitis virus (VEE), and Ross River virus.

Please add the following claims:

137. (New) The DNA molecule of claim 75, wherein non-cytopathicity is conferred by one or more mutations in the nsP2 gene of said replicase.

138. (New) The DNA molecule of claim 75, wherein temperature sensitivity is conferred by one or more mutations in the nsP4 gene of said replicase.

139. (New) The DNA molecule of claim 75, wherein non-cytopathicity is conferred by one or more mutations in the nsP2 gene of said replicase, and temperature sensitivity is conferred by one or more mutations in the nsP4 gene of said replicase.

140. (New) The DNA vector system of claim 103, wherein non-cytopathicity is conferred by one or more mutations in the nsP2 gene of said replicase.

141. (New) The DNA vector system of claim 103, wherein temperature sensitivity is conferred by one or more mutations in the nsP4 gene of said replicase.

142. (New) The DNA vector system of claim 103, wherein non-cytopathicity is conferred by one or more mutations in the nsP2 gene of said replicase, and temperature sensitivity is conferred by one or more mutations in the nsP4 gene of said replicase.

143. (New) The composition of claim 125, wherein non-cytopathicity is conferred by one or more mutations in the nsP2 gene of said replicase.

144. (New) The composition of claim 125, wherein temperature sensitivity is conferred by one or more mutations in the nsP4 gene of said replicase.

145. (New) The composition of claim 125, wherein non-cytopathicity is conferred by one or more mutations in the nsP2 gene of said replicase, and temperature sensitivity is conferred by one or more mutations in the nsP4 gene of said replicase.